

Enhancement of excess electron transfer efficiency in DNA containing a phenothiazine donor and multiple stable phenanthrenyl base-pairs

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Experimental section

General

All reactions were performed under argon in oven-dried glassware. Anhydrous solvents were obtained by filtration through activated aluminum oxide. Unless stated otherwise all chemicals and solvents were purchased from Sigma. Solvents for extractions and flash chromatography were distilled before use. Flash column chromatography (FCC) was performed using silica gel (230–400 mesh) from Silicycle. Thin layer chromatography was carried out on glass-backed plates precoated with silica gel (0.25mm, UV₂₅₄) from Macherey-Nagel. ¹H NMR was recorded at 300MHz or 400MHz on a Bruker AC-300 or a Bruker DRX-400. ¹³C NMR (75 MHz) was recorded on a Bruker AC-300. ³¹P NMR spectra were recorded at 121.4 MHz using 85% H₃PO₄ as an external standard. All spectra were referenced to the signals of the corresponding solvent. Chemical shifts are given in ppm (δ scale) and coupling constants (*J*) in Hz. High resolution electrospray ionization (ESI) mass spectra were recorded on a Thermo Scientific LTQ Orbitrap XL instrument. HPLC purification was performed using an Äkta™ basic 10/100 system (Amersham Pharmacia Biotech) equipped with a Phenomenex Jupiter semi-preparative RP-HPLC column (5μm, C18, 300Å).

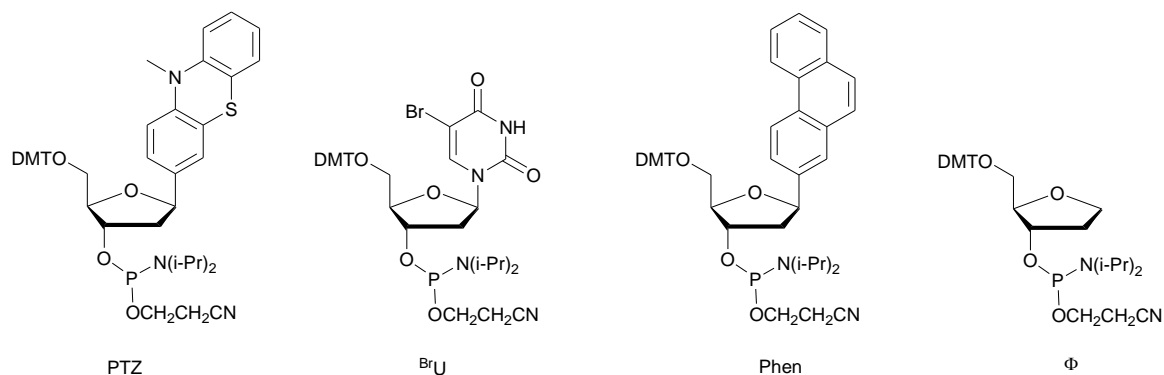
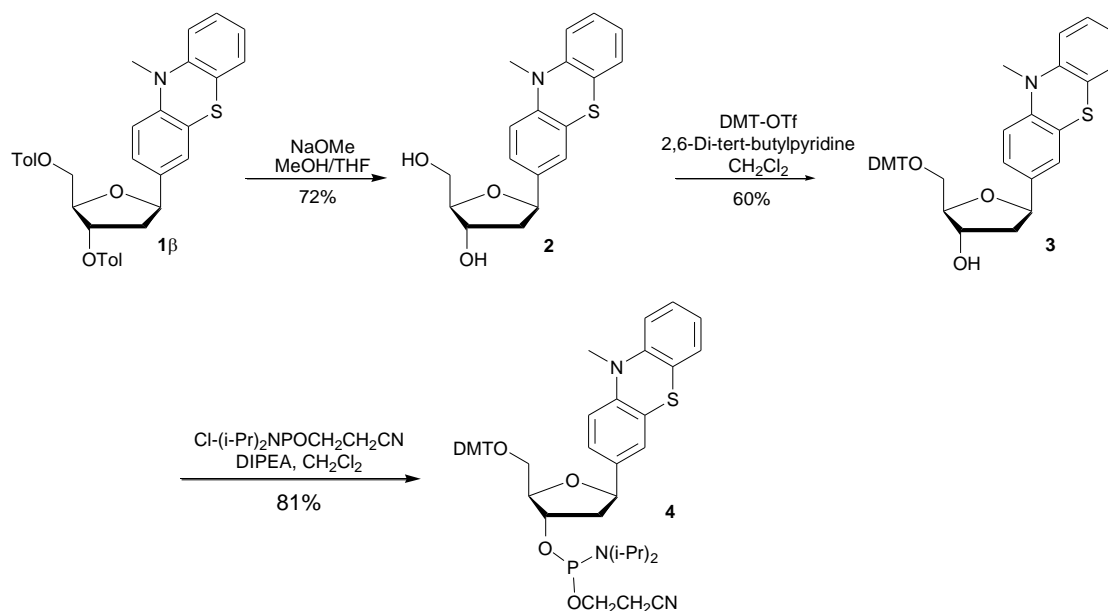


Figure S1. Chemical structures of phosphoramidites used in this study.



Scheme S1. Synthesis of a phenothiazine C-nucleoside analogue

Compound (2)

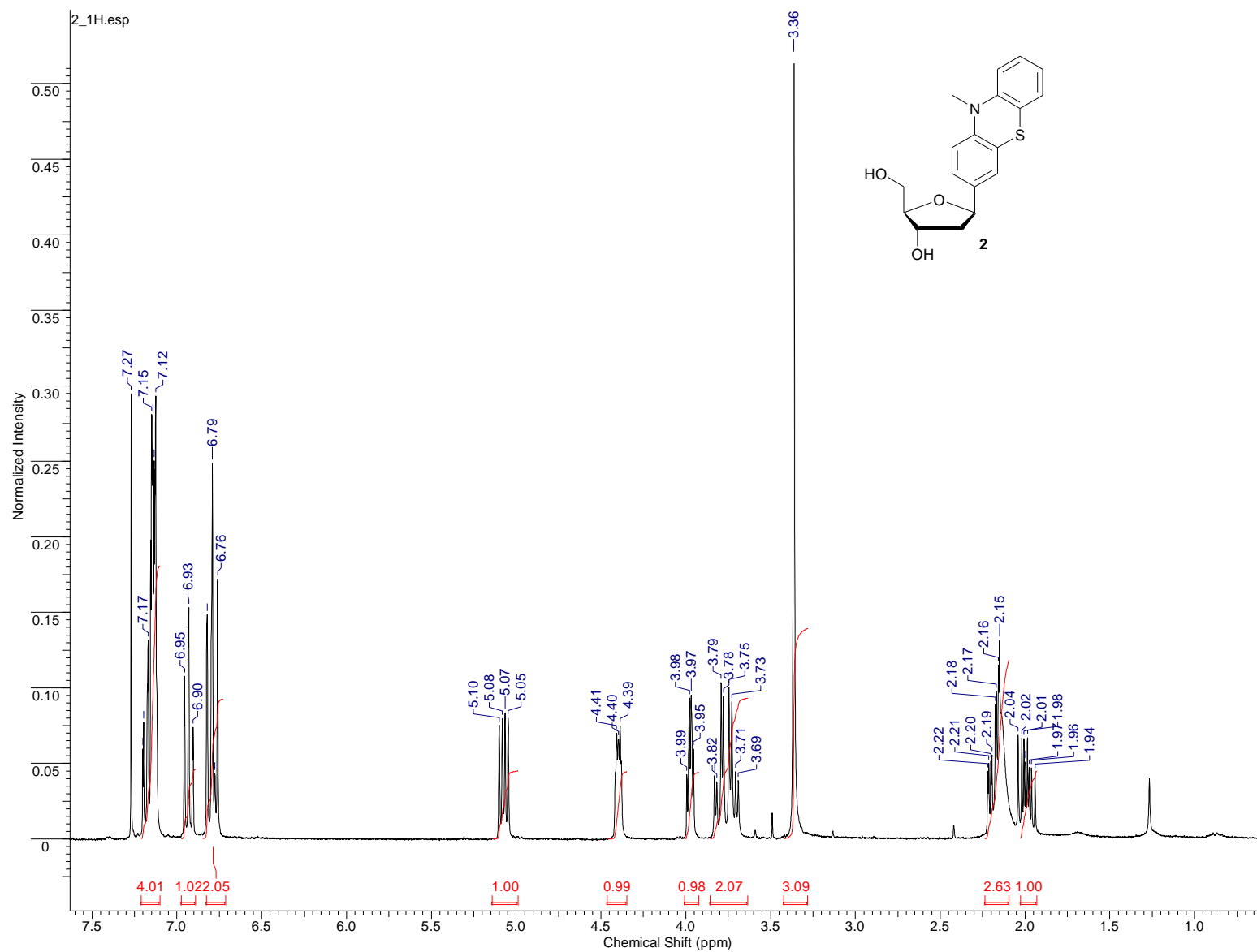
Compound **1β** (2.0 g, 3.5 mmol), was suspended in MeOH/THF (1:1, 40 mL), NaOMe (0.55 g, 10.2 mmol) was added and the reaction was stirred for 12 h. The mixture was concentrated under reduced pressure to approximately 5 mL, and purified by FCC, eluting with CHCl₃/MeOH (98:2 to 95:5) gave compound **2** (0.83 g, 72%). ¹H NMR (300MHz, CDCl₃): δ = 7.2 – 7.12 (m, 4H), 6.93 (t, *J* = 7.5, 1H), 6.79 (m, 2H), 5.08 (dd, *J* = 5.7, 10.2 Hz, 1H), 4.40 (m, 1H), 3.98 (m, 1H), 3.76 (m, 1H), 3.36 (s, 3H), 2.22 – 2.10 (m, 2H), 2.04 – 1.94 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 145.6, 135.2, 127.5, 127.1, 125.4, 124.8, 123.7, 123.1, 122.5, 114.0, 113.9, 87.2, 79.4, 73.7, 63.4, 43.7, 35.3. HRMS (ESI) *m/z* calcd for C₁₈H₂₀O₃NS [M⁺] 330.1158, found 330.1165

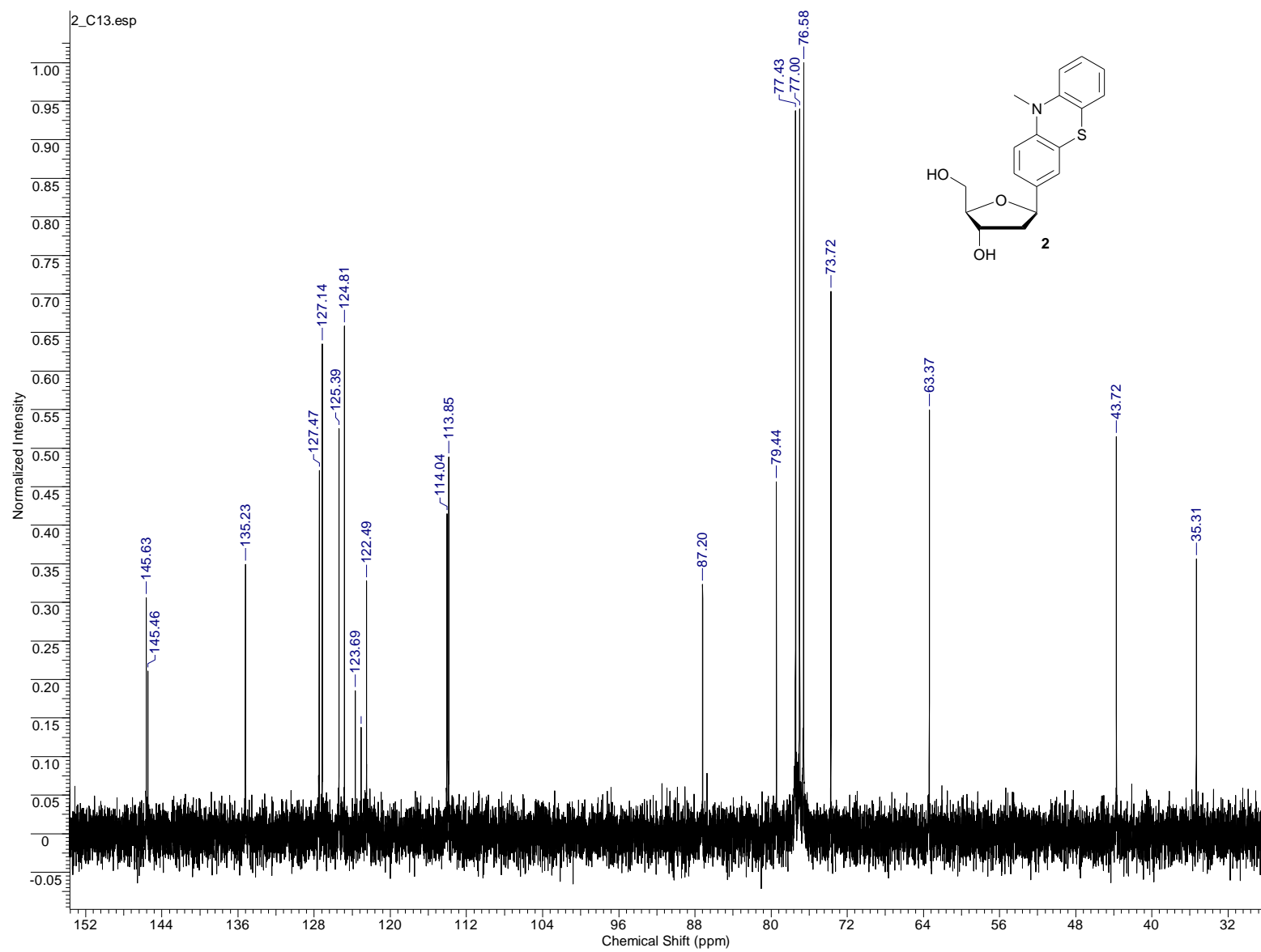
Compound (3)

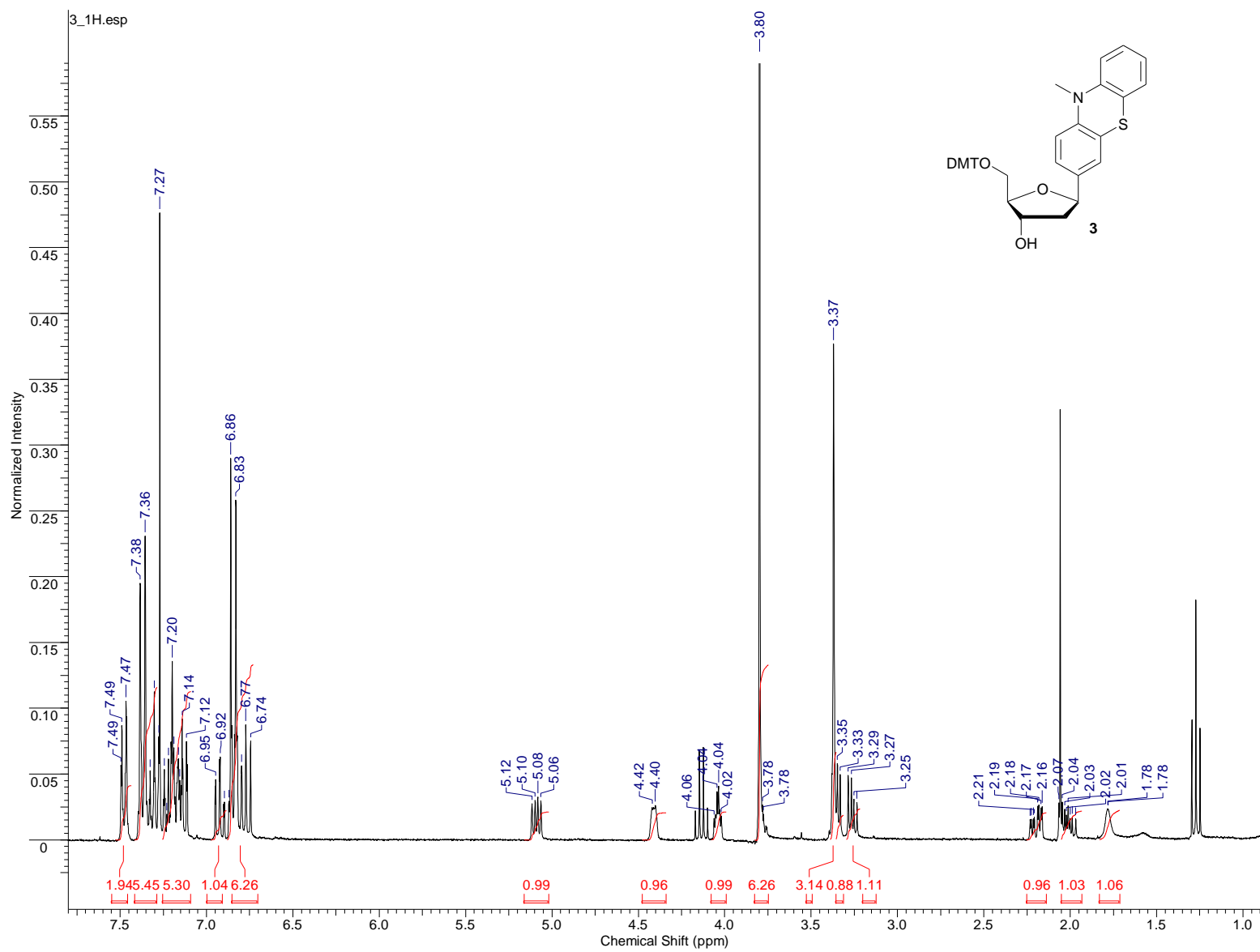
Compound **2** (645 mg, 2.0 mmol) was suspended in CH₂Cl₂ (25 mL), and cooled to 0 °C. To this suspension was added 2,6-di-tert-butyl-pyridine (0.82 g, 4.0 mmol) and DMT-triflate (1.1 g, 2.4 mmol) in portions. The reaction was stirred at 0 °C for 1 h., and stirred at rt until completion (4 h). The mixture was quenched with methanol (2 mL), diluted with CH₂Cl₂ and the organic phase was washed with saturated aqueous sodium bicarbonate, brine and dried over anhydrous MgSO₄. Purification by FCC, eluting with hexane/ethyl acetate (90:10 to 20:80 + ~1% NEt₃) gave **3** (760 mg, 60%). ¹H NMR (300MHz, CDCl₃): δ = 7.49 – 7.47 (m, 2H), 7.38 – 7.12 (m, 11H), 6.92 (t, *J* = 7.5, 1H), 6.87 – 6.74 (m, 6H), 5.09 (dd, *J* = 5.7, 10.2 Hz, 1H), 4.41 (m, 1H), 4.04 (m, 1H), 3.80 (s, 6H), 3.37 (s, 3H), 3.36 (s, 3H), 2.20 (m, 1H), 2.01 (m, 1H), 1.78 (br s, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 145.6, 135.2, 127.5, 127.1, 125.4, 124.8, 123.7, 123.1, 122.5, 114.0, 113.9, 87.2, 79.4, 73.7, 63.4, 43.7, 35.3. ¹³C NMR (75 MHz, CDCl₃): δ 158.4, 145.6, 145.0, 144.8, 136.0, 130.0, 128.1, 127.8, 127.3, 127.0, 126.7, 125.2, 124.7, 123.4, 123.2, 122.3, 113.9, 113.7, 113.1, 86.2, 86.1, 79.2, 77.4, 77.0, 76.6, 74.5, 64.5, 55.1, 43.7, 35.2. HRMS (ESI) *m/z* calcd for C₃₉H₃₇O₅NS [M⁺] 631.2387, found 631.2379

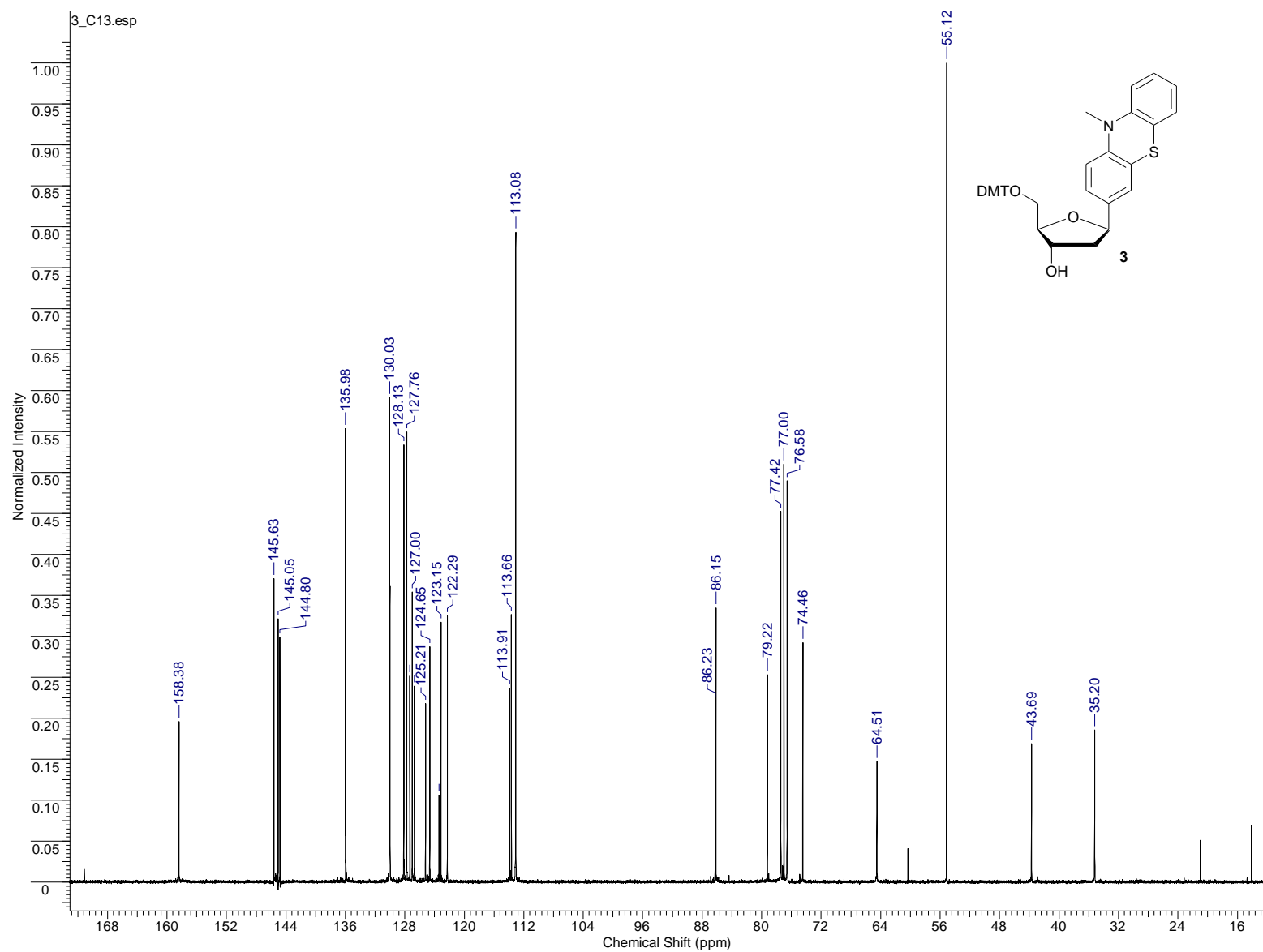
Compound (4)

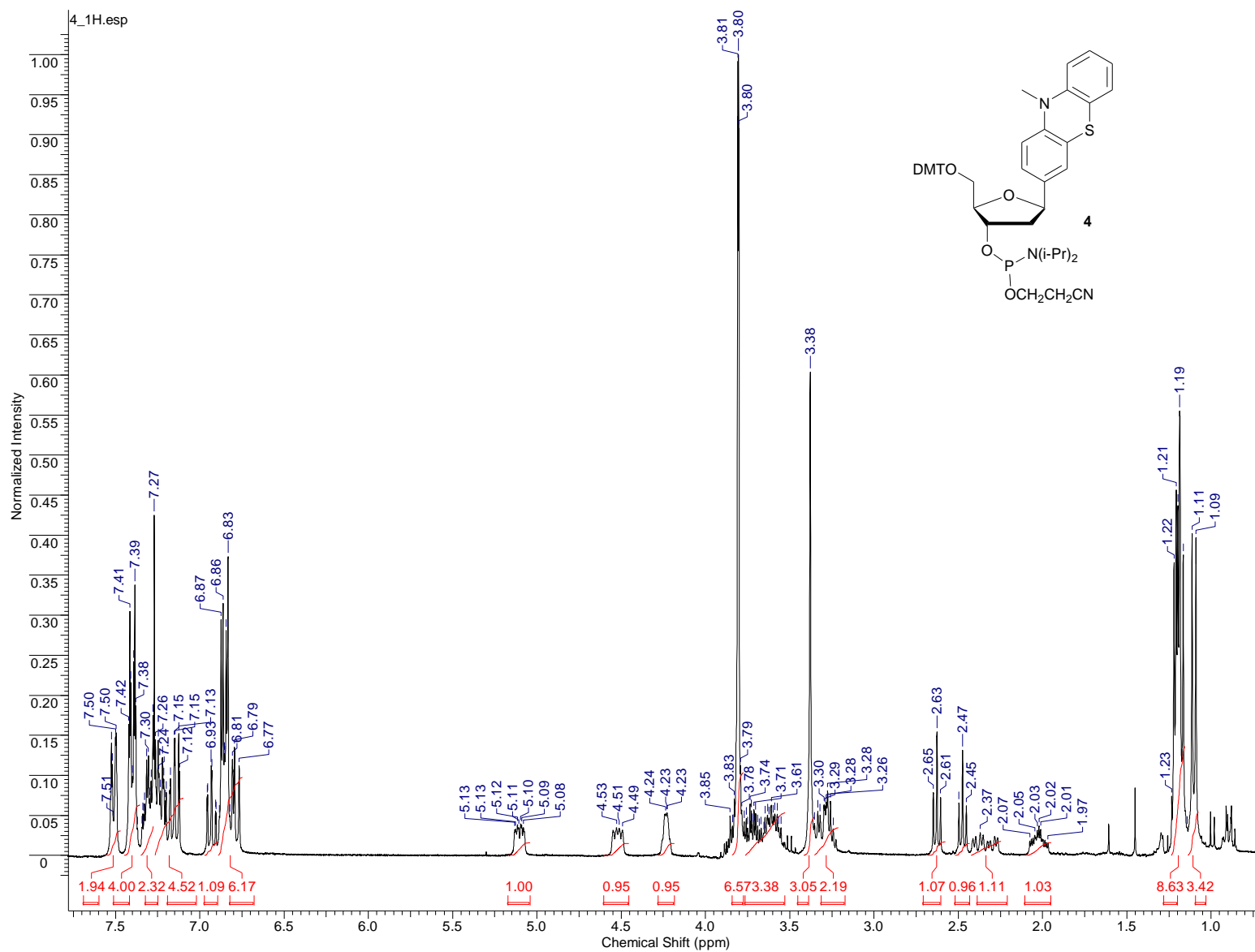
To a solution of compound **3** (675 mg, 1.1 mmol) in dry CH₂Cl₂ (15 mL), cooled to 0 °C, was added *N,N*-diisopropylethylamine (0.63 mL, 3.5 mmol), followed by 2-cyanoethyl diisopropylethylamine (320 µL, 1.4 mmol). The reaction was removed from the ice-bath and stirred for 1.0 h at rt, diluted with CH₂Cl₂ and the organic phase was washed with saturated aqueous sodium bicarbonate, brine and dried over anhydrous MgSO₄, purified by FCC, eluting with hexane/ethyl acetate (80:20 to 60:40 + ~1% NEt₃). The resulting oil was dissolved in a minimal amount of CH₂Cl₂ and precipitated by the addition of hexane (15 mL), the hexane was discarded and the residue was again dissolved in a minimal amount of CH₂Cl₂ and precipitated by the addition of hexane, the hexane was again discarded and the precipitated compound was dried under reduced pressure to give **4** (745 mg, 81%). ¹H NMR (300MHz, CDCl₃): δ = 7.52 – 7.50 (m, 2H), 7.42 – 7.38 (m, 4H), 7.33 – 7.12 (m, 7H), 6.92 (t, *J* = 7.4, 1H), 6.87 – 6.77 (m, 6H), 5.11 (m, 1H), 4.52 (m, 1H), 4.23 (m, 1H), 3.80 (m, 6H), 3.78 – 3.55 (m, 3 H), 3.36 (s, 3H), 3.33 – 3.24 (m, 2H), 2.63 (t, *J* = 6.8 Hz, 1 H), 2.47 (t, *J* = 6.6 Hz, 1 H), 2.41 – 2.25 (m, 1 H), 2.07 – 1.97 (m, 1H), 1.24 – 1.09 (m, 12 H). ¹³C NMR (75 MHz, CDCl₃): δ 158.4, 145.7, 145.2, 144.9, 136.0, 136.0, 130.1, 128.3, 128.2, 127.7, 127.3, 127.1, 126.7, 125.3, 124.8, 123.5, 123.3, 122.3, 113.9, 113.7, 113.1, 86.1, 85.6, 79.6, 79.6, 76.3, 76.1, 75.9, 75.6, 64.3, 64.2, 58.4, 58.2, 55.2, 43.3, 43.2, 43.1, 43.1, 35.3, 24.6, 24.5, 24.5, 24.37, 20.4, 20.3, 20.2, 20.1. ³¹P NMR (121.5 MHz, CDCl₃): δ 148.1, 147.9. HRMS (ESI) *m/z* calcd for C₄₈H₅₄N₃O₆PS [M⁺] 831.3465, found 831.3466.

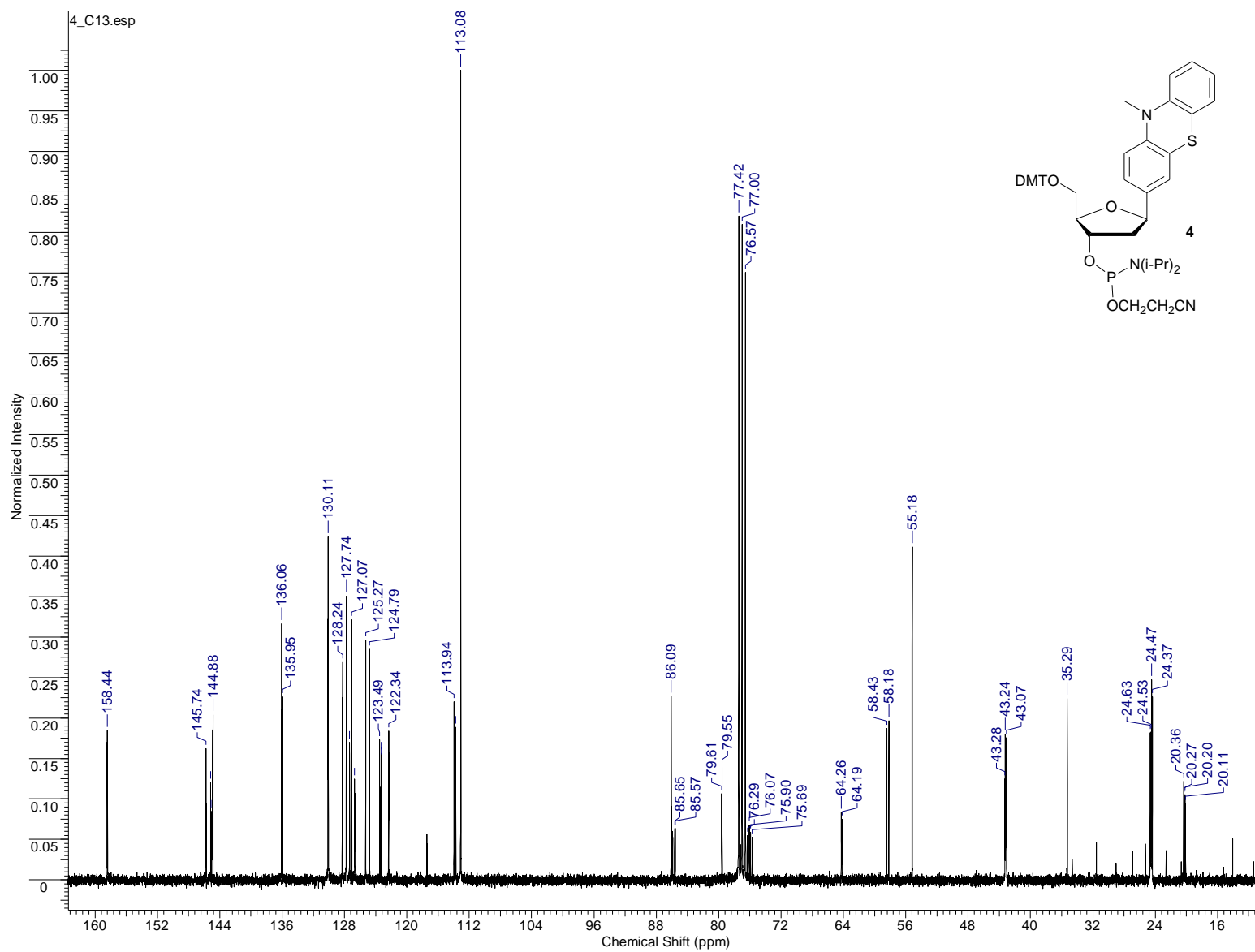


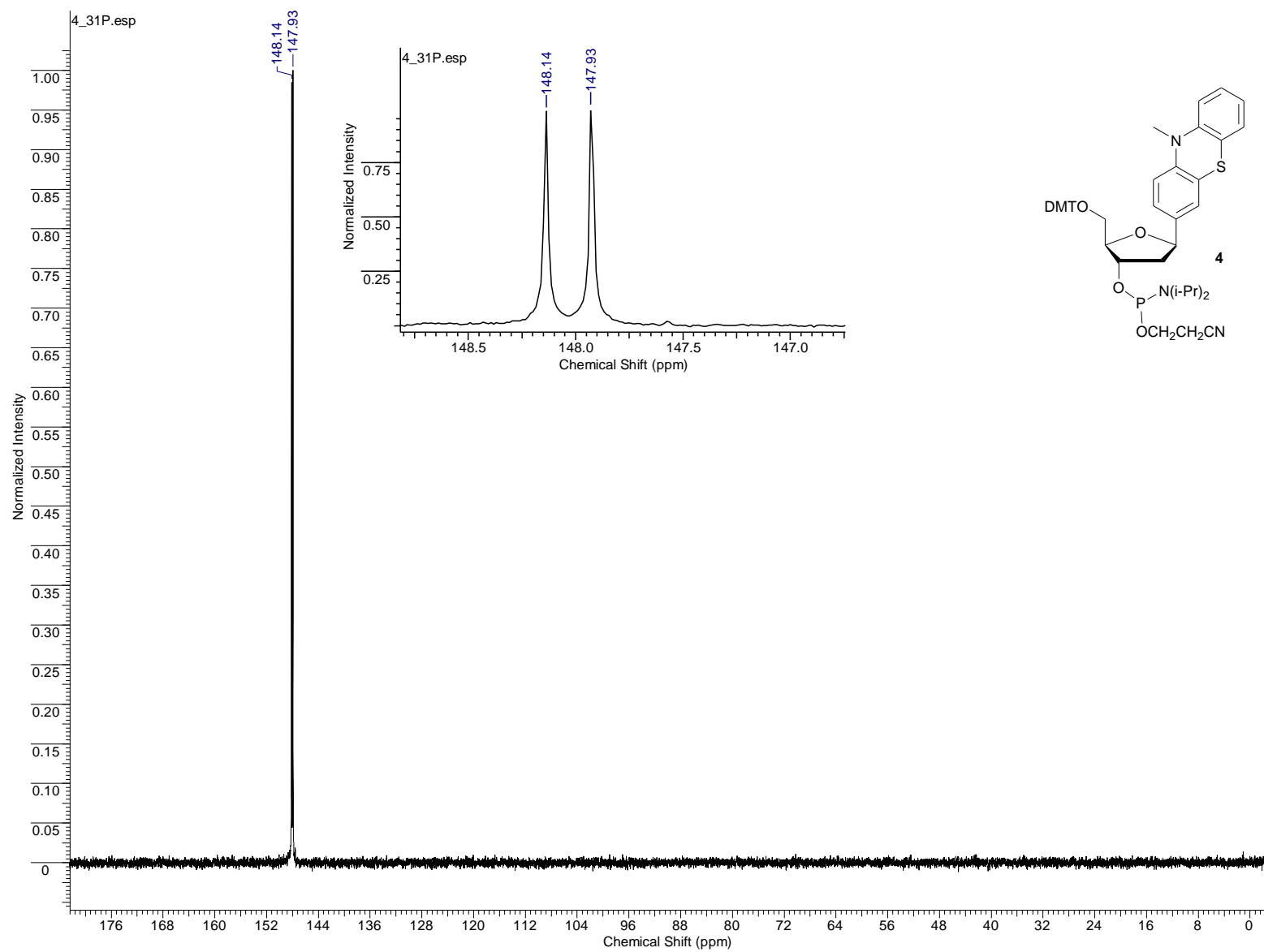












DNA synthesis

DNA synthesis was performed on a 1.3 μ mole scale on a Pharmacia Gene Assembler Plus DNA synthesizer following standard phosphoramidite protocols with 5-(ethylthio)-1H-tetrazole (0.25 M in CH_3CN) as the activator and coupling times of 6 min for the modified building blocks. Cleavage from the solid support and final deprotection was achieved with 30 % NH_4OH solution (55 $^\circ\text{C}$, 12 h). Sequences containing 5-bromouracil were deprotected with 30 % NH_4OH solution for 24 h at room temperature in order to avoid formation of 5-aminouracil. Purification was performed by RP-HPLC using A = 0.1 M triethylammonium acetate in H_2O , pH 7.0; B = 0.1 M triethylammonium acetate in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 4:1, pH 7.0, detection wavelength at 260 nm and 494 for fluorescein containing sequences. A flow rate of 3.5 ml/min was used. For oligonucleotides containing one Phen base surrogate a gradient from 0 - 35% B over 30 min was used, for oligonucleotides containing three Phen base surrogates a gradient from 0 - 45% B over 30 min was used.

UV Melting Curves

UV melting curves were recorded on a Varian Cary 100-Bio UV/VIS spectrophotometer (Varian Inc.), equipped with a Peltier element at 260 nm with a heating/cooling rate of 0.5 $^\circ\text{C}/\text{min}$. A cooling/heating cycle in the temperature range 10–90 $^\circ\text{C}$ was applied. T_m values were obtained from the maxima of the first derivatives of the melting curves using WinUV software. To avoid evaporation of the solution, the sample solutions were covered with a layer of dimethylpolysiloxane. All measurements were carried out in a buffer consisting of 10 mM Na_2HPO_4 , 150 mM NaCl, pH 7.0 at 1.2 μM strand concentration.

CD spectra

CD spectra were measured on a JASCO J-715 spectropolarimeter at the temperature indicated using quartz cuvettes with a path length of 1 cm. All measurements were carried out in a buffer consisting of 10 mM Na_2HPO_4 , 150 mM NaCl, pH 7.0 at 4.0 μM strand concentration.

Figure S2. CD spectra of Duplexes **D1-D9**.

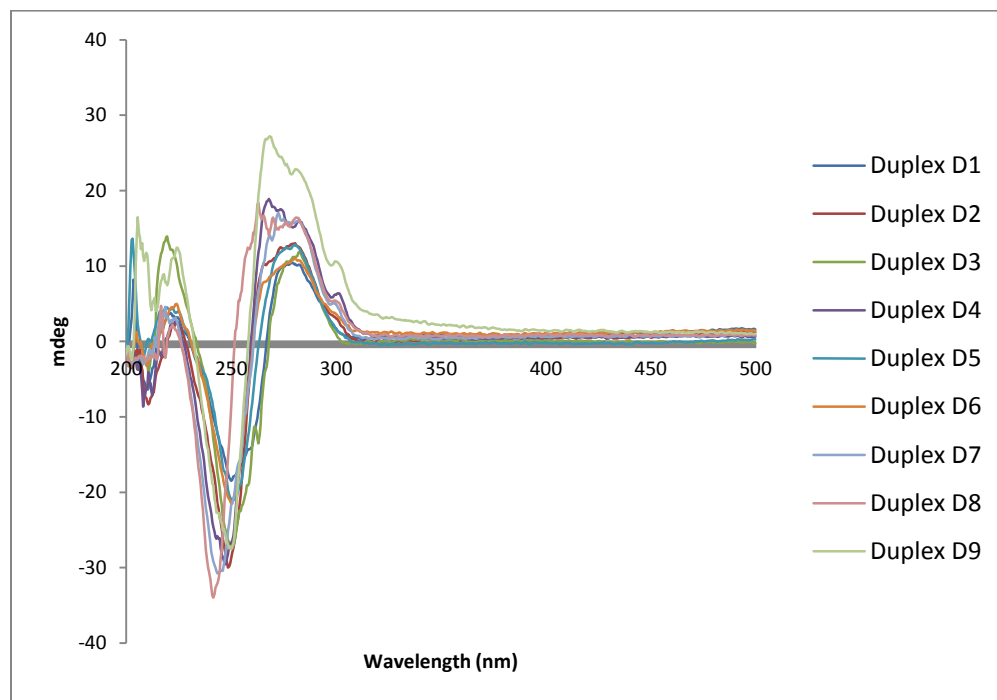


Table S1. MS data of single strands **1-14** and fragments **a** and **b**. ^bss denotes the single stranded oligonucleotide.

ss ^b	Sequence	m/z calcd	m/z found
1	5'-GCGATΦAAATGCG-3'	3874.5	3874.2
2	3'-CGCTAPtzT ^{Br} UTACGC-Fluo	4590.0	4589.9
3	5'-GCGATΦPhenAATGCG-3'	3917.6	3917.3
4	3'-CGCTAPtzPhen ^{Br} UTACGC-Fluo	4642.2	4641.7
5	5'-GCGATΦAAAAATGCG-3'	4500.9	4500.6
6	3'-CGCTAPtzTTT ^{Br} UTACGC-Fluo	5198.4	5198.1
7	5'-GCGATΦPhenPhenPhenAATGCG-3'	4630.2	4630.0
8	3'-CGCTAPtzPhenPhenPhen ^{Br} UTACGC-Fluo	5354.7	5354.1
9	5'-GCGATAPhenAATGCG-3'	4050.7	4050.2
10	3'-CGCTATPhen ^{Br} UTACGC-Fluo	4555.0	4554.5
11	3'-CGCTAPtzPhenTTACGC-Fluo	4577.3	4576.6
12	5'-GCGATAPhenPhenPhenAATGCG-3'	4763.3	4762.7
13	3'-CGCTATPhenPhenPhen ^{Br} UTACGC-Fluo	5267.6	5266.8
14	3' CGCTAPtzPhenPhenPhenTTACGC-Fluo	5289.93	5289.4
a	3'-O ₄ P-ACGC-Fluo	1775.3	1775.7
b	3'-O ₄ P-TACGC-Fluo	2079.4	2079.8

Irradiation

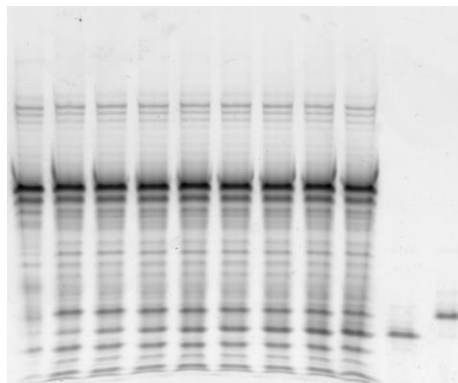
The irradiation sample was prepared by mixing a duplex (4 μ M, 10 mM Na-P_i-buffer, 150 mM NaCl, pH 7.0) in equimolar solutions of the single strands together and heating them to 90 °C for 5 min. in the dark, cooling to room temperature over night. Samples were deoxygenated by bubbling N₂ for 30 min. through the solution and then irradiated in quartz glass cuvettes (1 cm). Cuvettes with fresh prepared duplexes were placed 6 cm in front of a Sylvania Blacklight (F8 8W T5 BL350 G5) equipped with a glass cut-off filter (wall thickness 3 mm, \leq 300nm). The cuvettes were ice cooled to maintain the temperature of the irradiated sample at 4 °C. Aliquots (60 μ l) of the sample solution (1000 μ l) were taken after 0, 5, 10, 15, 20, 30, 40, 60 min of irradiation, placed into sterile a container and stored protected from light at room temperature. After withdrawing the last aliquot, all samples were treated with piperidine (6.6 μ l) and subsequently heated to 90 °C for 30 min., cooled to RT and lyophilized.

Gels

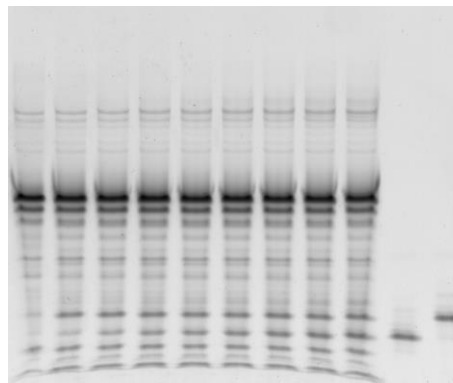
Samples were dissolved in loading buffer (60 μ l; 900 μ l of formamide, 100 μ l EDTA solution pH 8.0) and treated with ultrasound for 3 min. at RT. After sonication samples were heated to 60 °C for 10 min. and loaded (20 μ l) onto a gel (19:1 acrylamide-bisacrylamide, 20 % w/v, 8 M urea, 100 mM Tris-borate and 20 mM EDTA buffer, pH 8.3). Gels were run at 250 V for 2 h and analyzed using a FLA-3000 Phosphor Imager (Fujifilm). Band intensities were quantified with ImageJ. Yields were determined by dividing the specific fragment area by the total area, where the aliquot without piperidine treatment served as a blank. All experiments were performed in triplicates.

Figure S3. Fluorescence images of 20 % denaturing PAGEs of duplexes **D1-D9** and single strands **2, 4, 6** and **8**. Lane 1 represents aliquot without irradiation and piperidine treatment. Lane 2-9 are aliquots withdrawn after 0, 5, 10, 15, 20, 30, 40, 60 min of irradiation and treated with 10 % piperidine at 90 °C for 30 min. Lane 10 represents the specific strand scission fragment **a** as a reference.

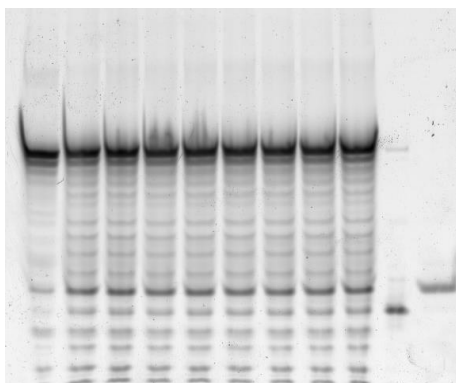
Duplex **D1**



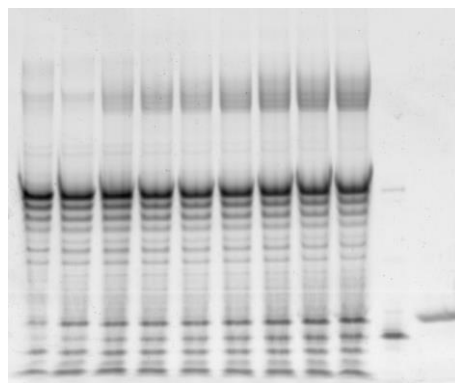
Duplex **D2**



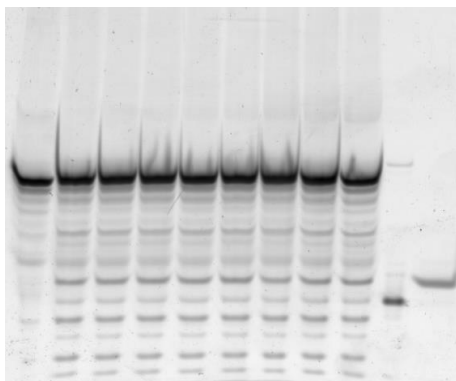
Duplex **D3**



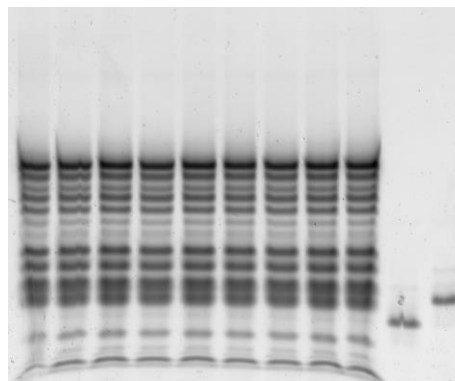
Duplex **D4**



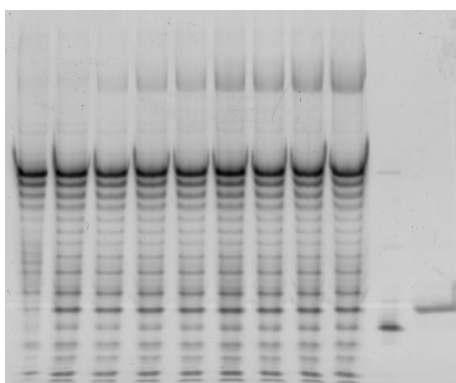
Duplex **D5**



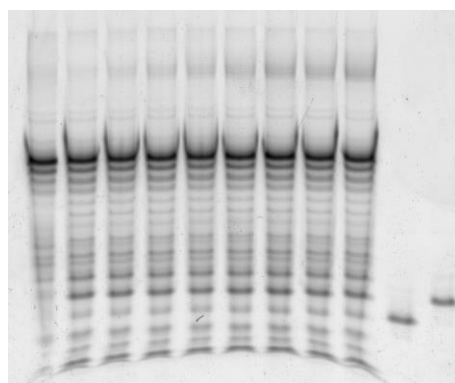
Duplex **D6**



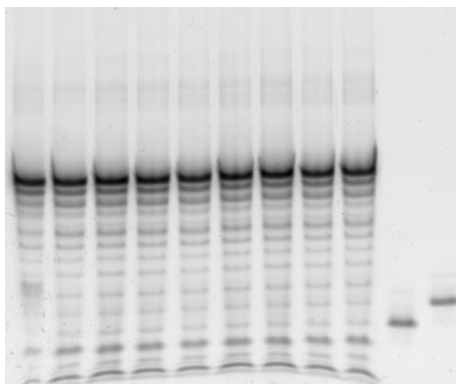
Duplex **D7**



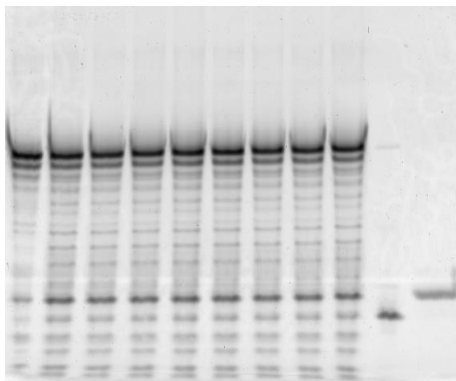
Duplex **D8**



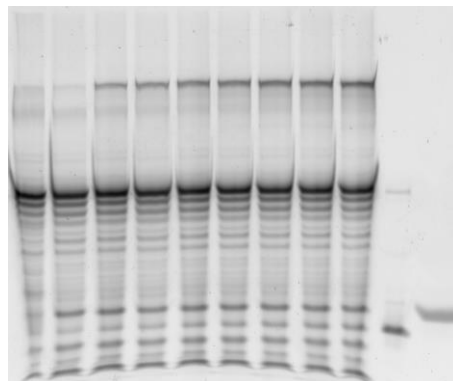
Duplex **D9**



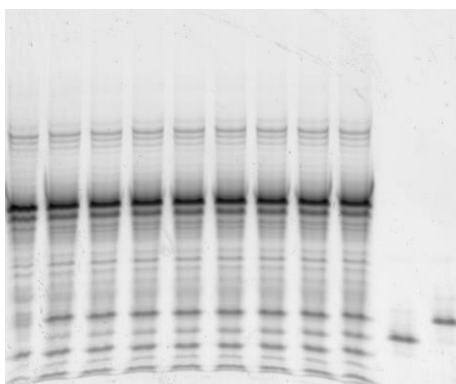
Single strand **6**



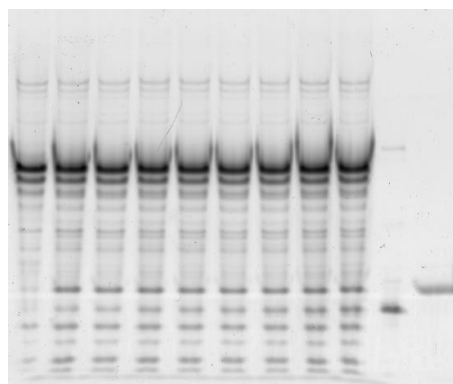
Single strand **8**



Single strand **2**



Single strand **4**



Intra-strand photoaddition

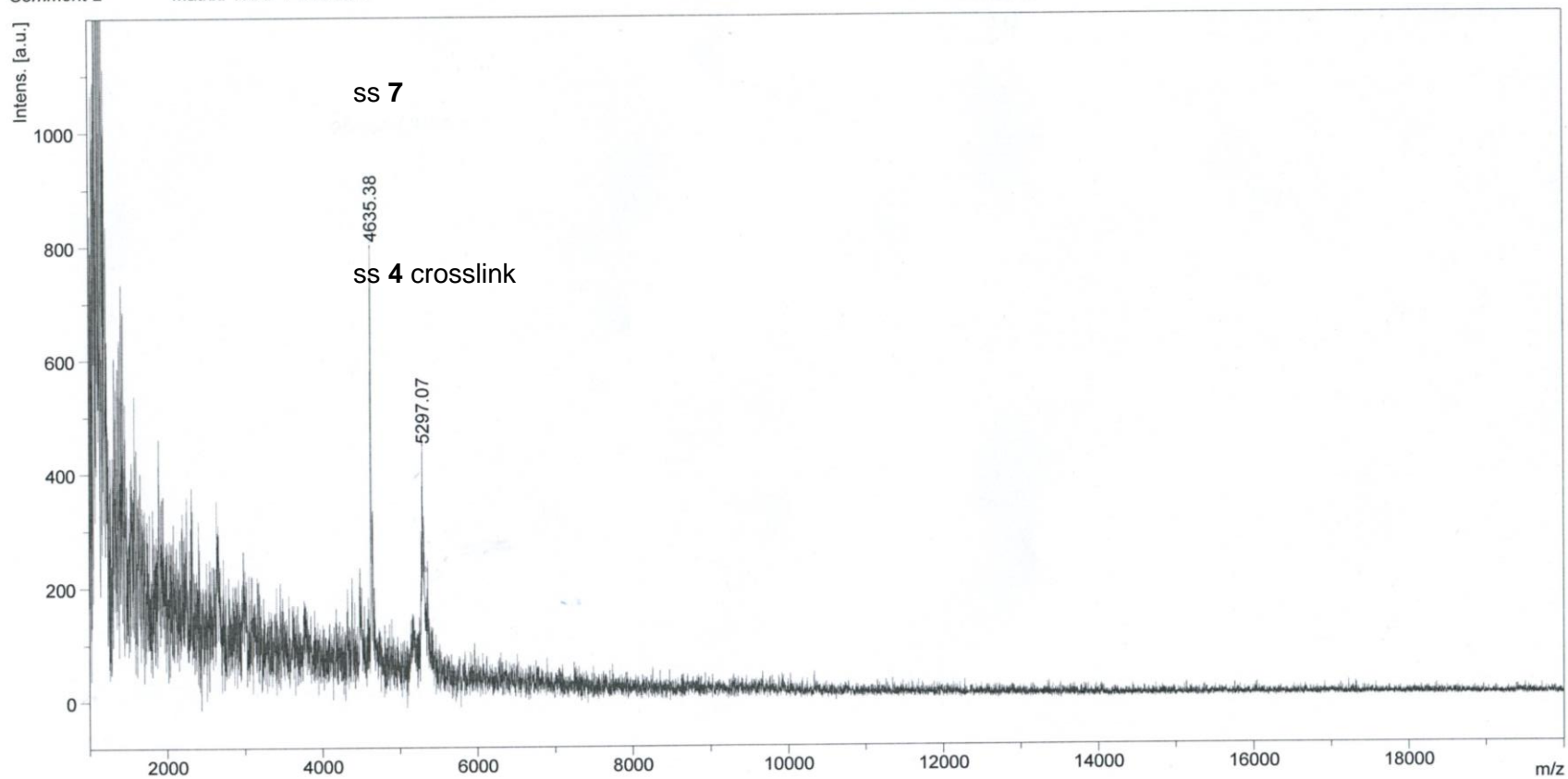
Duplex **2** (1 mL, 4 μ M, 10 mM Na-P_i-buffer, 150 mM NaCl, pH 7.0) was deoxygenated by bubbling N₂ for 30 min. through the solution and then irradiated in quartz glass cuvettes (1 cm). Cuvettes with fresh prepared duplex was placed 6 cm in front of a Sylvania Blacklight (F8 8W T5 BL350 G5) equipped with a

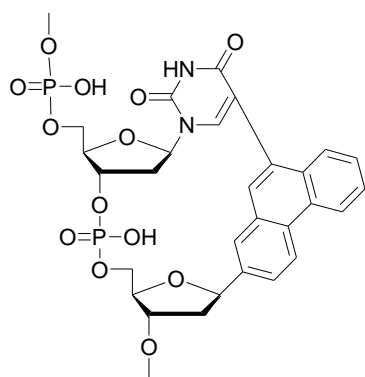
glass cut-off filter (wall thickness 3 mm, < 300nm) and irradiated for 1h at 0 °C. The sample was transferred into a sterile container, lyophilized, desalted by sep-pak column and separated by RP-HPLC using A = 0.1 M triethylammonium acetate in H₂O, pH 7.0; B = 0.1 M triethylammonium acetate in CH₃CN/H₂O 4:1, pH 7.0, with a detection wavelength at 260 nm and 494. A flow rate of 1ml/min. and a gradient of 0 - 80% B over 50 min was used. The purified fragment was characterized by MALDI and NSI⁺ mass spectroscopy.

ss **4** crosslink (m/z [M+Na⁺+3H⁺] found 5297.07; calcd. 5296.98)

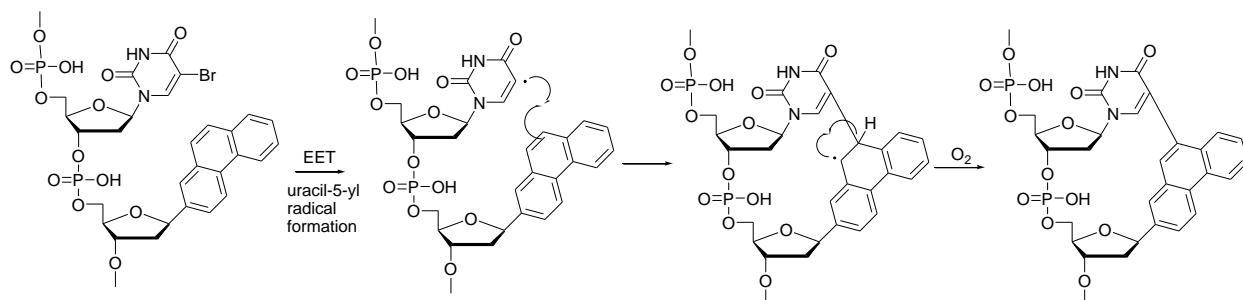
ss **7** (m/z [M+4H⁺] found 4635.38; calcd. 4634.2)

Comment 1 DNA CL E8
Comment 2 Matrix: THAP : TAC 2 : 1

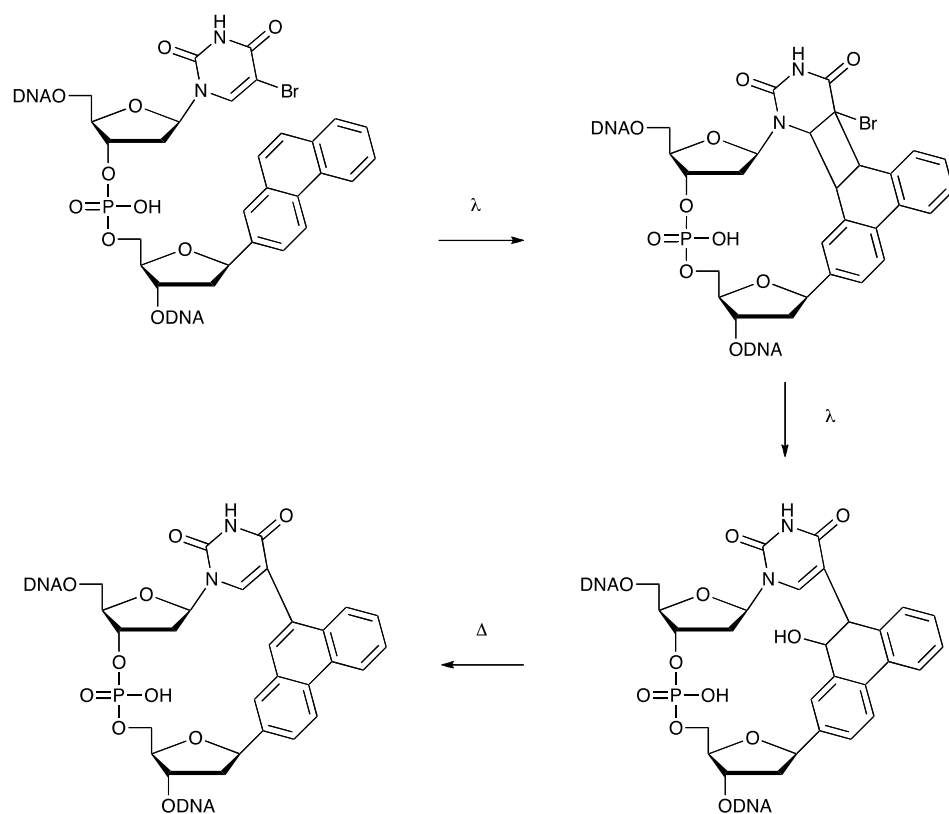




Scheme S2. Proposed structure of the Phenanthrene-uridinyl Adduct



Scheme S3. Proposed radical mechanism for the phenanthrene-uridinyl Adduct formation occurring by the formation of the uracil-5-yl



Scheme S4. Alternative cross-linking mechanism, initiated by a photochemical induced [2+2] cycloaddition reaction of ^{Br}U and a phenanthrenyl unit. This mechanism seems less likely as it does not occur with duplexes **D6** and **D9** containing a dT instead of a ^{Br}U unit.